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IV. TRANSMISSION OF TULARÆMIA BY THE BEDBUG, CIMEX LECTULARIUS.

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The experiments here reported show that the bedbug *Cimex lectularius*, which commonly infests beds and bites human beings, is capable of transmitting tularæmia from an infected to a healthy white mouse. Two distinctly different methods of transmission were successful. In one method we followed the usual procedure of first allowing the insects to feed on an infected animal and then to feed on a healthy animal. In the other method we followed the first half of the usual procedure, but the second half was reversed; i. e., the mouse ate the infected bug instead of the infected bug biting the mouse. Following the usual method, transmission was successful in 10 experiments in which the intervals which elapsed between biting the infected mice and biting the healthy mice were a few seconds, 18 hours, 7 days, 15 days, and 71 days. Following the unusual method, transmission was successful in 55 experiments in which the intervals which elapsed between the bug's biting the infected mouse and the mouse's eating the infected bugs varied uniformly from 6 hours to 100 days; this latter limit promises to be still further extended and to keep pace with the natural length of life of a bedbug.

METHODS EMPLOYED.

White mice and guinea pigs were the experimental animals used. Infection was due to strain (S) of *Bacterium tularense* isolated in 1920 from a human case of tularæmia in Utah. The bedbugs were collected from cracks in the wooden cages in which the laboratory stock of guinea pigs and white mice are bred. The bugs were kept on hand from one to three weeks before attempting to infect them. They were confined at all times to test tubes. They sucked an infecting meal of blood either from the tail of an inoculated white

[1] See Public Health Reports, vol. 36, No. 30, July 29, 1921, pp. 1731-1753.

mouse, the tail being poked into the tube through a hole in the gauze which covered the mouth of the tube, or they sucked through the gauze, the tube being inverted and held on the infected mouse's abdomen.

The white mice which were used for infecting the bugs were inoculated subcutaneously on the back with the heart's blood of a dead infected mouse.

Bugs were applied to a sick mouse between 72 and 96 hours after inoculation, since four days was the maximum life of a white mouse inoculated subcutaneously.

The tubes containing the bugs were always kept at room temperature, which during the summer was practically that of the outside air, but after cool weather arrived in October it was that of the ordinary steam-heated laboratory, which averaged 22° C.

A tube contained not over 40 bugs and was supplied with a strip of filter paper on which the bugs rested and deposited their feces. The white mice were kept in glass battery jars, one mouse to each jar.

TRANSMISSION FOLLOWING BEDBUG BITES.

Ten white mice died with typical lesions of tularæmia following bedbug bites. Their histories are summarized at the head of the table and given more in detail in their appropriate places in the body of the table. Experimental transmission by biting is largely dependent upon interrupted feeding. An engorged bug will not bite. A partially engorged bug will bite. Forced interruption of a bug's meal of blood on the infected animal conduces to a completion of that meal on the healthy animal. The shorter the interruption the greater the likelihood of transmission. The lengths of interruption in our experiments were (1) a few seconds, (2) 18 hours, (3) 48 hours, (4) no interruption.

(1) *Interruption of a few seconds.*—The bugs were allowed to feed 2½ minutes on the infected mouse and then, after only a second's interval, they were allowed to feed for 2½ minutes on the healthy mouse; they fed in this manner alternately on the infected and healthy mice during 15 minutes, thus permitting of three insertions of the proboscis in each of the two animals. It is not probable that all of the bugs made six insertions, but certainly each bug made multiple insertions. In this method transmission was probably accomplished purely by a grossly contaminated proboscis.

During an experiment the infected mouse and the healthy mouse were tied to a board, abdomens upward. The abdomen of the infected mouse was shaved three days previously, but the abdomen of the healthy mouse was unshaved and unclipped. The bugs were contained in a glass tube, the mouth of which was covered with gauze. The mouth of the tube was held alternately in contact with

the two abdomens, the bugs biting through the gauze. No evidences of defecation were noted on the hair of the healthy mice.

Results: Five sets of bugs, averaging 25 bugs to each set, bit four infected mice, and then, after an interval of only a few seconds, bit 5 healthy mice according to the above-mentioned interrupted method of feeding. Transmission occurred in all instances. The intervals which elapsed between biting the healthy mice and their deaths from tularæmia were 3½, 4½, 4½, 5½, and 5½ days, the average being 4½ days. There were no unsuccessful attempts at transmission when the period of interruption was only a few seconds.

(2) *Interruption of 18 hours.*—The bugs were allowed to feed on the abdomen of the infected mouse for only 2½ minutes, after which they were set aside for 18 hours, at the end of which time they were applied for 12½ minutes in contact with the tail of a healthy mouse and allowed to feed to full engorgement. For this purpose the tail of the healthy mouse was poked through a hole in the gauze which covered the mouth of the tubes. No evidences of defecation were noted on the tails of the healthy mice while being bitten by the bugs.

Results: Three sets of bugs, averaging 70 bugs to each set, fed for 2½ minutes on three infected mice, and then after an interval of 18 hours, fed to engorgement on three healthy mice. Transmission occurred in two instances, the intervals which elapsed between biting the healthy mice and their deaths from tularæmia being 4½ and 5½ days. The mouse bitten by the third set of bugs remained well. (See table, Lots 262, 263, and 264.)

(3) *Interruption of 48 hours.*—Five sets of bugs, averaging 54 bugs to each set, fed for 2½ minutes on 5 infected mice and then, after an interval of 48 hours, fed to full engorgement on 5 healthy mice. Transmission occurred in no instance. (See table, Lots 272 to 284.)

(4) *Noninterrupted feeding.*—This method of transmission varied from the foregoing methods in that the bugs' first meal was a full meal. They were allowed to feed on the abdomen or tail of the infected mouse to full engorgement without interruption.

Having sucked one full meal of infected blood, all subsequent feedings took place on healthy mice and were for the purpose of determining whether infection of healthy mice would follow the bites of infected bugs.

The second, third, and fourth feedings took place approximately on the third, sixth, and tenth days after the infecting meal. Subsequent feedings occurred approximately every 10 days throughout the life of the bugs.

While feeding on healthy mice, the bugs were applied in contact with the tails of these mice for periods of one hour, thus not only permitting them to feed to engorgement, but encouraging them to defecate on the tails. For this purpose the tail of the healthy mouse

was kept poked for one hour into a slender glass tube containing the bugs. The bugs avoid resting on the tail of the mouse if possible; they even prefer glass to a mouse's tail as a resting place. In order to compel them to rest on the tail, the caliber of the glass tube was just enough larger than the diameter of the tail to permit the passage of the bugs along the tail, and, moreover, the tube was held in a vertical position so that the bugs were forced to rest on the tail of the mouse. The result was that the bugs deposited their feces principally on the tail so that the points of biting must have become overlaid with bug feces in some instances.

Results: Ten lots of bugs (Nos. 230 to 258), varying from 16 to 140 bugs, but averaging 88 bugs to the lot, fed to engorgement on 10 infected mice, and then, after intervals of from 3 to 110 days, fed to engorgement during one hour on the tails of 23 healthy mice, feeding an average of five times on each healthy mouse. Transmission occurred in 3 out of 23 mice. The intervals which elapsed between biting the infected mice and biting the 3 healthy mice were 7, 15, and 71 days, respectively, and the intervals between biting the 3 healthy mice and their deaths from tularæmia were 6, 5, and 7 days, respectively; the number of bugs employed in the three transmissions were 28, 24, and 14, respectively.

TRANSMISSION OF TULARÆMIA DUE TO EATING INFECTED BEDBUGS.

Transmission experiments with bedbugs and white mice which do not take into account the mouse's habit of eating bedbugs will be full of errors. White mice readily attack and eat living bedbugs; they eat dead bedbugs with equal readiness. If the bugs are infected with *Bacterium tularense*, the mouse is almost certain to die of tularæmia. One of our white mice confined with 45 infected living bugs within a small glass jar free from bedding or other hiding places for the bugs, ate all the bugs in less than an hour, being at the rate of one bug a minute. A mouse ate 15 living infected bugs over night, the mouse and bugs being loose in a glass battery jar containing bedding composed of coarse screenings of sawdust. Two healthy mice, dropped into a large jar containing cut hay and small wooden boxes and a plentiful supply of bugs which had been infected 21 days previously, died of tularæmia 3½ and 5½ days after entering the jar. Presumably the mice contracted the infection from eating the bugs rather than from the bites of the bugs.

Experiments in other fields of research designed to demonstrate transmission by fleas often have permitted the unrestricted presence of rodents and fleas in the same container. Such a procedure in bug-mouse experiments would leave the experimenter totally in the dark on the question of whether transmission resulted from the bedbug's biting the mouse or the mouse's eating the bedbug.

We conducted 72 bug-eating experiments, 55 of which were successful. The infected bugs came from 10 lots (Nos. 230 to 258). These bugs had fed once to engorgement on infected mice and were fed subsequently every 3 to 10 days on healthy mice. These healthy mice served to test the infectiveness of the bug bites. Some of the bugs were found dead every morning, while others were dying and consequently almost motionless. These infected dead or dying bugs were fed to mice, thereby infecting the mice. The same lots of bugs, therefore, were used in the bug-biting and bug-eating experiments.

Bugs which were to be eaten were dropped, together with a white mouse, into a bottomless glass cylinder 4 inches in diameter which rested on a blotter; the purpose of the blotter was to absorb the urine. The mouse remained until he had eaten the bugs, which in some instances was overnight. The mouse was then transferred to a glass battery jar, commonly used as a mouse cage, each mouse occupying a separate jar.

Summary.—Seventy-two white mice ate dying or dead bugs from each of 10 lots of infected bugs. Of this number, 55 died from tularæmia and 17 remained well. The number of bugs offered to and eaten by each mouse varied from 1 to 10, the average being 3. The average length of time which elapsed between eating infected bugs and death of the mice from tularæmia was 4½ days. Of 20 mice which each ate one infected bug, 14 died acutely from tularæmia. The average length of time from the date of infection of the 14 bugs until they were eaten was 65 days. Three white mice which each ate a bug infected 100 days previously died five, four, and five days later from tularæmia.

Bacterium tularense suffers no apparent diminution of virulence by reason of long residence in the bedbug.

It is the intention to continue these eating experiments throughout the life of the bugs, which promises to be long.

INFECTIVITY OF FRESH BEDBUG FECES.

While transmission experiments with our 10 lots of infected bugs were in progress, the infectivity of the bug feces was frequently tested. The strips of filter paper on which the bugs habitually rested served also for the deposition of feces. When the strips were renewed, the old ones were soaked in saline solution, rubbed in a mortar, strained through gauze, and the suspension was either injected subcutaneously into a guinea pig or mixed with corn meal and fed to a white mouse. From each of ten lots of infected bugs two samples of feces were collected about 10 days apart and between 17 and 58 days after the date of infection of the bugs. Both samples from a given lot of bugs were fed at the time of collection to the same mouse. Ten mice

which were thus fed with fresh feces from 10 lots of infected bugs remained well.

Forty-one samples of feces, representing about three samples from each of the 10 lots of infected bugs, were injected, while fresh, subcutaneously into 41 guinea pigs. At least one sample in each of the 10 lots was collected 35 days or more after the date on which bugs were infected, and in one instance the sample was collected 120 days after the date of infection. The 41 guinea pigs all died acutely from tularæmia.

The two sets of experiments show in marked contrast the invariable susceptibility of the guinea pig's subcutaneous tissue to the infected bedbug feces in question, and the invariable resistance of the mouse's stomach to the same material.

Summary.—The fresh feces of bedbugs which were infected with *Bacterium tularense* by sucking the blood of infected white mice and which were fed every 10 days thereafter on the blood of healthy white mice, contained virulent organisms of this infection at all times and did so up to 120 days after the date of infection of the bugs, and probably will do so throughout the life of the bugs.

INFECTIVITY OF DRIED FECES OF BEDBUGS.

Feces deposited by our 10 lots of infected bugs on filter papers, between 46 and 75 days after the dates of infection of the bugs, were subsequently set aside and allowed to remain on those filter papers in a dried condition at an average room temperature of 20° C. unexposed to direct light. At the end of 20 days of drying, the filter papers were soaked in saline solution, ground in a mortar, and the pooled suspension of feces was injected subcutaneously into two guinea pigs, causing their deaths within six days with typical lesions of tularæmia.

Feces deposited on filter papers by 8 lots of infected bugs (see table, Lots 262 to 284) between 18 and 70 days after the dates of infection of the bugs, were subsequently kept under the conditions described above for 25 days, at the end of which time the pooled suspension was injected subcutaneously into a guinea pig, causing its death 8 days later with typical lesions of tularæmia.

TRANSMISSION TO GUINEA PIGS.

The foregoing experiments relate to transmission to white mice. A few attempts were made on transmission to guinea pigs by infected bedbugs. Bugs from 6 of our 10 lots of infected bugs bit 6 guinea pigs, respectively. The guinea pigs were exposed to the bugs under the following conditions.

A healthy guinea pig and infected living bugs were dropped into a bottomless glass cylinder, 6 inches in diameter, which rested on a blotter; the purpose of the blotter was to absorb the urine. Guinea pig and bugs remained in the cylinder overnight. The guinea pigs never showed any tendency to eat the bugs. The bugs were plainly seen to feed to engorgement on the feet of the guinea pigs. They never crawled over the guinea pig's feet but approached only close enough to insert their outstretched proboscides.

The bugs were counted into the cylinder in the evening and counted out in the morning. The evening and morning counts always tallied, with one exception. The guinea pigs all remained well, with the exception of one (see Lot 258). This one was the guinea pig in whose cylinder we failed to recover one bug one morning, 47 having been counted in and only 46 having been counted out. In this case the blotter had been chewed to small bits. The presumption is that the guinea pig unintentionally swallowed one infected bug in chewing its blotter, because it died 7 days later from tularæmia and showed five typical cervical buboes and a chain of typical lymph glands in the mesentery, in addition to the ordinary typical lesions of the spleen and liver.

In order to test the susceptibility of guinea pigs to infection by eating, five lots of 2 bugs each were fed to engorgement on 5 infected mice and 24 hours later were fed to five guinea pigs, respectively, each pair of bugs being ground in a mortar with normal saline solution and applied to a small piece of bread which the guinea pigs readily ate. One of the five guinea pigs died seven days after eating the two infected bugs and showed the typical lesions of tularæmia; the other four remained well. This is taken as strong evidence that the one guinea pig which died from tularæmia after confinement with 46 infected bedbugs contracted its infection from the accidental ingestion of the one missing bug rather than from the bites of the bugs.

In another experiment pooled fresh feces from our 10 lots of infected bugs were fed on bread to a guniea pig with negative results.

Summary.—An average of 35 infected bedbugs from each of 6 of our 10 lots of infected bugs were exposed with 6 healthy guinea pigs, respectively, during approximately the third, sixth, and tenth nights after the date of infection of the bugs. Although freely bitten by the bugs, only 1 of the guinea pigs contracted tularæmia, and in this instance infection is believed to have taken place from the ingestion of a missing infected bug (see Lot No. 258).

Acknowledgment: The determination of specimens of *Cimex lectularius* were made by Dr. H. E. Ewing, of the Bureau of Entomology, Department of Agriculture.

GENERAL SUMMARY.

The common bedbug *Cimex lectularius* transmitted tularæmia from infected to healthy mice in 10 instances, in which the intervals which elapsed between biting the infected and biting the healthy mice were a few seconds, 18 hours, 7 days, 15 days, and 71 days. The exact parts played by bites and by feces in the 10 transmissions are impossible of determination.

White mice readily eat living and dead bugs.

White mice which eat infected bugs usually contract tularæmia. Of 20 white mice which each ate one infected bug, 14 died acutely from tularæmia. The average length of time from the date of infection of the 14 bugs until they were eaten was 65 days. Three white mice which each ate a bug infected 100 days previously died 5, 4, and 5 days later from tularæmia.

Guinea pigs apparently do not eat bugs intentionally.

Guinea pigs bitten by infected bugs failed to contract tularæmia, with one exception; in the latter instance the guinea pig probably ate one infected bug unintentionally and thereby contracted the infection.

The fresh feces of bedbugs which were infected with *Bacterium tularense* by sucking the blood of infected white mice, and which were fed every 10 days thereafter on the blood of healthy white mice, contained virulent organisms of this infection at all times and did so up to 120 days after the date of infection of the bugs.

Feces of infected bedbugs deposited on filter papers at least 46 days after the dates of infection of the bugs and subsequently dried for 20 days contained virulent organisms of *Bacterium tularense* at the end of that time.

In spite of the last two preceding paragraphs, the fresh feces of infected bugs have always failed to infect white mice or guinea pigs which ate those feces.

Bacterium tularense suffered no apparent diminution of virulence by reason of long residence in bedbugs. This virulence was manifested by acute death from tularæmia within seven, five, five, and six days, respectively, in cases of—

(1) A mouse which was bitten by bugs infected 71 days previously;

(2) A mouse which ate a bug infected 100 days previously;

(3) A guinea pig which was infected with fresh feces of bugs infected 120 days previously; and

(4) A guinea pig injected with bug feces which had been deposited on filter paper at least 46 days after the date of infection of the bugs and which had subsequently dried on filter paper at an average room temperature of 20° C. for 20 days.

Various lots of bugs; each lot was infected by biting a separate mouse.	*Infectivity of bug feces:* Number of days after date of infection of bugs when their fresh feces were (1) injected into a guinea pig or (2) fed to a white mouse. "Positive" means death from tularæmia.	*Transmission due to eating infected bugs:* Length of time after date of infection of bugs when dying or dead bugs were eaten by a white mouse. "Positive" means death from tularæmia.	*Transmission following bug bites:* Length of time after date of infection of bugs when bugs were allowed to bite a healthy white mouse or guinea pig. "Positive" means death from tularæmia.	Remarks.
	(The 10 "Positives" following bedbug bites are assembled in brief at the right of the table for ready reference.)		Few seconds; 25 bugs bit abdomen of mouse. Positive. Few seconds; 30 bugs bit abdomen of mouse. Positive. Few seconds; 12 bugs bit abdomen of mouse. Positive. Few seconds; 30 bugs bit abdomen of mouse. Positive. Few seconds; 30 bugs bit abdomen of mouse. Positive. 18 hours; 62 bugs bit tail of mouse. Positive. 18 hours; 67 bugs bit tail of mouse. Positive. 7 days; 28 bugs bit tail of mouse. Positive. 15 days; 24 bugs bit tail of mouse. Positive. 71 days; 14 bugs bit tail of mouse. Positive.	See Lot No. 244. See Lot No. 248. See Lot No. 248. See Lot No. 250. See Lot No. 252. See Lot No. 263. See Lot No. 264. See Lot No. 258. See Lot No. 258. See Lot No. 234.
Lot No. 244. 80 bugs infected Sept. 15, 1921.	(1) Feces injected: 13 days. Positive. 23 days. Positive. 43 days. Positive. (2) Feces fed: 22 days. Negative. 35 days. Negative.	6 hours; fed 2 dying bugs to mouse 82. Positive. 6 hours; fed 2 dying bugs to mouse 83. Positive 2 days; fed 6 dead bugs to mouse 84. Positive. 35 days; fed 5 dying bugs to mouse 85. Positive. 44 days; fed 5 dead bugs to mouse 86. Negative. 55 days; fed 5 dead bugs to mouse 87. Positive. 67 days; fed 2 dead bugs to mouse 99. Positive. 74 days; fed 1 dead bug to mouse 100. Positive.	Few seconds; 25 bugs bit abdomen of mouse 60. Positive. 8 days; 16 bugs bit tail of mouse 5. Negative. 13 days; 30 bugs bit tail of mouse 5. 15 days; 28 bugs bit tail of mouse 5. 18 days; 26 bugs bit tail of mouse 5. 23 days; 22 bugs bit tail of mouse 6. Negative. 27 days; 20 bugs bit tail of mouse 6. 35 days; 18 bugs bit tail of mouse 6. 39 days; 16 bugs bit tail of mouse 6. 54 days; 14 bugs bit tail of mouse 6. 74 days; 4 bugs bit tail of mouse 6. 84 days; 3 bugs bit tail of mouse 6.	Reported above.
Lot No. 248. 140 bugs infected Sept. 19, 1921.	(1) Feces injected: 20 days. Positive. 30 days. Positive. 40 days. Positive. (2) Feces fed: 22 days. Negative. 35 days. Negative.	6 hours; fed 2 dying bugs to mouse 10. Positive. 6 hours; fed 2 dying bugs to mouse 11. Negative. 6 hours; fed 2 dying bugs to mouse 12. Negative. 6 hours; fed 2 dying bugs to mouse 13. Negative. 31 days; fed 9 dead bugs to mouse 14. Negative. 32 days; fed 10 dying bugs to mouse 15. Negative. 37 days; fed 10 dead bugs to mouse 16. Positive. 84 days; fed 6 dead bugs to mouse 101. Negative.	Few seconds; 30 bugs bit abdomen of mouse 8. Positive. Few seconds; 12 bugs bit abdomen of mouse 9. Positive. 2 days; 20 bugs bit tail of mouse 14. Negative. 8 days; 30 bugs bit tail of mouse 14. 11 days; 25 bugs bit tail of mouse 14. 14 days; 24 bugs bit tail of mouse 14. 18 days; 22 bugs bit tail of mouse 14. 20 days; 21 bugs bit tail of mouse 14. 30 days; 50 bugs bit tail of mouse 15. Negative. 40 days; 36 bugs bit tail of mouse 15. 50 days; 24 bugs bit tail of mouse 15. 8 days; 48 bugs bit feet of guinea pig 2. Negative. 11 days; 45 bugs bit feet of guinea pig 2. 13 days; 43 bugs bit feet of guinea pig 2.	Reported above. Do.

Transmission of tularæmia by bedbugs of the species Cimex lectularius—Continued.

Various lots of bugs; each lot was infected by biting a separate mouse.	*Infectivity of bug feces:* Number of days after date of infection of bugs when their fresh feces were (1) injected into a guinea pig or (2) fed to a white mouse. "Positive" means death from tularæmia.	*Transmission due to eating infected bugs:* Length of time after date of infection of bugs when dying or dead bugs were eaten by a white mouse. "Positive" means death from tularæmia.	*Transmission following bug bites:* Length of time after date of infection of bugs when bugs were allowed to bite a healthy white mouse or guinea pig. "Positive" means death from tularæmia.	Remarks.
Lot No. 250. 90 bugs infected Sept. 23, 1921.	(1) Feces injected: 17 days. Positive. 26 days. Positive. 36 days. Positive. (2) Feces fed: 17 days. Negative. 31 days. Negative.	6 hours; fed 2 dying bugs to mouse 18. Positive. 6 hours; fed 2 dying bugs to mouse 19. Positive. 6 hours; fed 2 dying bugs to mouse 20. Positive. 28 days; fed 8 dead bugs to mouse 21. Positive. 30 days; fed 5 dying bugs to mouse 22. Positive. 33 days; fed 5 dead bugs to mouse 23. Positive. 36 days; fed 5 dead bugs to mouse 24. Positive. 56 days; fed 1 dead bug to mouse 88. Positive. 67 days; fed 1 dead bug to mouse 102. Negative.	Few seconds; 31 bugs bit abdomen of mouse 17. Positive. 3 days; 45 bugs bit feet of guinea pig 3. Negative. 6 days; 43 bugs bit feet of guinea pig 3. 9 days; 41 bugs bit feet of guinea pig 3. 14 days; 35 bugs bit tail of mouse 25. Negative. 17 days; 33 bugs bit tail of mouse 25. 26 days; 14 bugs bit tail of mouse 25. 36 days; 8 bugs bit tail of mouse 25. 46 days; 7 bugs bit tail of mouse 25. 56 days; 6 bugs bit tail of mouse 25. 66 days; 4 bugs bit tail of mouse 25. 76 days; 4 bugs bit tail of mouse 25.	Reported above.
Lot No. 252. 86 bugs infected Sept. 22, 1921.	(1) Feces injected: 16 days. Positive. 27 days. Positive. 37 days. Positive. (2) Feces fed: 17 days. Negative. 27 days. Negative.	1 day; fed 2 dying bugs to mouse 26. Positive. 1 day; fed 2 dying bugs to mouse 27. Positive. 1 day; fed 2 dying bugs to mouse 28. Positive. 27 days; fed 4 dead bugs to mouse 29. Positive. 30 days; fed 5 dying bugs to mouse 30. Negative. 35 days; fed 6 dead bugs to mouse 31. Positive. 44 days; fed 3 dead bugs to mouse 32. Positive. 57 days; fed 1 dead bug to mouse 89. Positive. 77 days; fed 1 dying bug to mouse 91. Positive.	Few seconds; 30 bugs bit abdomen of mouse 33. Positive. 5 days; 45 bugs bit tail of mouse 34. Negative. 7 days; 41 bugs bit tail of mouse 34. 10 days; 36 bugs bit tail of mouse 34. 16 days; 34 bugs bit tail of mouse 35. Negative. 20 days; 32 bugs bit tail of mouse 35. 27 days; 28 bugs bit tail of mouse 36. Negative. 37 days; 17 bugs bit tail of mouse 36. 47 days; 9 bugs bit tail mouse 36. 57 days; 9 bugs bit tail of mouse 36 67 days; 8 bugs bit tail of mouse 36. 77 days; 7 bugs bit tail of mouse 36.	Reported above.
Lot No. 258. 130 bugs infected Sept. 29, 1921.	(1) Feces injected: 7 days. Positive. 24 days. Positive. 41 days. Positive. (2) Feces fed: 19 days. Negative. 29 days. Negative.	6 hours; fed 2 dying bugs to mouse 49. Positive. 12 hours; fed 2 dying bugs to mouse 50. Positive. 2 days; fed 2 dying bugs to mouse 51. Positive. 27 days; fed 8 dead bugs to mouse 52. Positive. 29 days; fed 5 dead bugs to mouse 53. Positive. 32 days; fed 4 dead bugs to mouse 54. Positive. 34 days; fed 2 dead bugs to mouse 55. Negative. 36 days; fed 1 dying bug to mouse 56. Positive.	36 hours; 47 bugs bit feet of guinea pig 6. Positive. 3 days; 40 bugs bit feet of guinea pig. 6. 7 days; 28 bugs bit tail of mouse 63. Positive. 15 days; 21 bugs bit tail of mouse 64. Positive. 19 days; 20 bugs bit tail of mouse 65. Negative. 29 days; 20 bugs bit feet of mouse 65. 40 days; 13 bugs bit tail of mouse 65. 15 days; 58 bugs bit tail of mouse 66. Negative.	Reported above. Do.

		36 days; fed 1 dying bug to mouse 57. Positive. 36 days; fed 1 dying bug to mouse 58. Positive. 37 days; fed 1 dead bug to mouse 59. Negative. 40 days; fed 3 dead bugs to mouse 61. Positive. 54 days; fed 1 dying bug to mouse 92. Positive.	19 days; 47 bugs bit tail of mouse 66. 29 days; 35 bugs bit feet of mouse 66. 40 days; 28 bugs bit feet of mouse 66. 50 days; 25 bugs bit tail of mouse 66. 60 days; 16 bugs bit tail of mouse 96. Negative. 70 days; 16 bugs bit tail of mouse 97. Negative.	
Lot No. 230. 112 bugs infected Sept. 1, 1921.	(1) Feces injected: 28 days. Positive. 30 days. Positive. 33 days. Positive. 38 days. Positive. 44 days. Positive. 54 days. Positive. 71 days. Positive. 80 days. Positive. 90 days. Positive. 100 days. Positive. 110 days. Positive. 120 days. Positive. (2) Feces fed: 48 days. Negative. 58 days. Negative.	16 days; fed 45 living bugs to mouse 78. Positive. 28 days; fed 20 living bugs to mouse 79. Positive. 53 days; 4 dying bugs to mouse 80. Positive. 57 days; fed 5 dead bugs to mouse 81. Positive. 71 days; fed 2 dead bugs to mouse 82. Positive. 83 days; fed 1 dead bug to mouse 93. Positive. 160 days; fed 1 dying bug to mouse 94. Positive. 163 days; fed 1 dead bug to mouse 105. Negative.	28 days; 38 bugs bit tail of mouse 1. Negative. 30 days; 38 bugs bit tail of mouse 1. 28 days; 28 bugs bit tail of mouse 2. Negative. 30 days; 28 bugs bit tail of mouse 2. 33 days; 33 bugs bit tail of mouse 2. 44 days; 28 bugs bit tail of mouse 3. Negative. 50 days; 27 bugs bit tail of mouse 3. 54 days; 21 bugs bit tail of mouse 3. 69 days; 16 bugs bit tail of mouse 3. 80 days; 15 bugs bit tail of mouse 3. 90 days; 14 bugs bit tail of mouse 3. 100 days; 12 bugs bit tail of mouse 3.	
Lot No. 234. 60 bugs infected Sept. 8, 1921.	(1) Feces injected: 1 day. Positive. 6 days. Positive. 10 days. Positive. 14 days. Positive. 20 days. Positive. 30 days. Positive. 50 days. Positive. (2) Feces fed: 36 days. Negative. 46 days. Negative.	43 days; fed 5 dying bugs to mouse 77. Negative. 67 days; fed 1 dead bug to mouse 95. Negative. 160 days; fed 1 dead bug to mouse 103. Positive. 160 days; fed 1 dead bug to mouse 104. Positive.	1 day; 60 bugs bit tail of mouse 4. 3 days; 34 bugs bit tail of mouse 4. 6 days; 36 bugs bit tail of mouse 4. 10 days; 27 bugs bit tail of mouse 4. 20 days; 26 bugs bit tail of mouse 4. 31 days; 25 bugs bit tail of mouse 4. 36 days; 24 bugs bit tail of mouse 4. 42 days; 21 bugs bit tail of mouse.4. 46 days; 18 bugs bit tail of mouse 4. 61 days; 15 bugs bit tail of mouse 4. 71 days; 14 bugs bit tail of mouse 4. Positive. 80 days; 14 bugs bit tail of mouse 5. Negative. 90 days; 14 bugs bit tail of mouse 5.	Reported above.
Lot No. 247. 16 bugs infected Sept. 19, 1921.	(1) Feces injected: 21 days. Positive. 30 days. Positive. 40 days. Positive. (2) Feces fed: 26 days. Negative. 35 days. Negative.	15 days; fed 3 dead bugs to mouse 75. Positive. 51 days; fed 1 dead bug to mouse 76. Positive.	2 days; 14 bugs bit feet of guinea pig 1. Negative. 7 days; 14 bugs bit feet of guinea pig 1. 10 days; 13 bugs bit feet of guinea pig 1. 13 days; 13 bugs bit feet of guinea pig 1. 18 days; 7 bugs bit tail of mouse 7. Negative. 21 days; 7 bugs bit tail of mouse 7. 30 days; 7 bugs bit tail of mouse 7. 40 days; 5 bugs bit tail of mouse 7. 50 days; 5 bugs bit tail of mouse 7. 60 days; 4 bugs bit tail of mouse 7. 70 days; 4 bugs bit tail of mouse 7. 80 days; 3 bugs bit tail of mouse 7.	

Transmission of tularæmia by bedbugs of the species Cimex lectularius—Continued.

Various lots of bugs; each lot was infected by biting a separate mouse.	*Infectivity of bug feces:* Number of days after date of infection of bugs when their fresh feces were (1) injected into a guinea pig or (2) fed to a white mouse "Positive" means death from tularæmia.	*Transmission due to eating infected bugs:* Length of time after date of infection of bugs when dying or dead bugs were eaten by a white mouse. "Positive" means death from tularæmia.	*Transmission following bug bites:* Length of time after date of infection of bugs when bugs were allowed to bite a healthy white mouse or guinea pig. "Positive" means death from tularæmia.	Remarks.
Lot No. 254. 44 bugs infected Sept. 25, 1921.	(1) Feces injected: 20 days. Positive. 35 days. Positive. (2) Feces fed: 30 days. Negative. 40 days. Negative.	1 day; fed 3 dead bugs to mouse 37. Positive. 1 day; fed 3 dead bugs to mouse 38. Positive. 31 days; fed 5 dying bugs to mouse 39. Negative. 35 days; fed 1 dead bug to mouse 40. Negative. 53 days; fed 1 dead bug to mouse 98. Negative.	3 days; 28 bugs bit feet of guinea pig 4. Negative. 5 days; 24 bugs bit feet of guinea pig 4. 8 days; 23 bugs bit feet of guinea pig 4. 16 days; 30 bugs bit tail of mouse 41. Negative. 20 days; 21 bugs bit tail of mouse 41. 29 days; 14 bugs bit tail of mouse 41. 34 days; 9 bugs bit tail of mouse 41. 44 days; 8 bugs bit tail of mouse 41. 54 days; 6 bugs bit tail of mouse 41. 64 days; 6 bugs bit tail of mouse 41. 74 days; 6 bugs bit tail of mouse 41.	
Lot Nos. 255–256. 126 bugs infected Sept. 26, 1921.	(1) Feces injected: 14 days. Positive. 24 days. Positive. 35 days. Positive. (2) Feces fed: 15 days. Negative. 27 days. Negative.	1 day; fed 3 dying bugs to mouse 42. Positive. 1 day; fed 3 dying bugs to mouse 43. Positive. 27 days; fed 14 dead bugs to mouse 44. Positive. 30 days; fed 5 dying bugs to mouse 45. Positive. 40 days; fed 4 dead bugs to mouse 46. Positive. 54 days; fed 1 dead bug to mouse 90. Positive.	3 days; 50 bugs bit feet of guinea pig 5. Negative. 6 days; 46 bugs bit feet of guinea pig 5. 3 days; 50 bugs bit tail of mouse 47. Negative. 6 days; 50 bugs bit tail of mouse 47. 12 days; 48 bugs bit tail of mouse 48. Negative. 14 days; 45 bugs bit tail of mouse 48. 28 days; 17 bugs bit tail of mouse 48. 42 days; 6 bugs bit tail of mouse 48. 52 days; 6 bugs bit tail of mouse 48. 62 days; 5 bugs bit tail of mouse 48. 72 days; 5 bugs bit tail of mouse 48.	
Lot No. 263. 62 bugs infected Oct. 4, 1921.	Feces injected: 35 days. Positive.		18 hours; 62 bugs bit tail of mouse 67. Positive.	Reported above.
Lot No. 264. 62 bugs infected Oct. 4, 1921.	35 days. Positive.		18 hours; 67 bugs bit tail of mouse 68. Positive.	Reported above.

Lot No. 262. 80 bugsinfected Oct. 2, 1921.	35 days. Positive.		18 hours; 80 bugs bit tail of mouse 69. Negative.	
Lot No. 272. 66 bugsinfected Oct. 11, 1921.	30 days. Positive.		48 hours; 66 bugs bit tail of mouse 70. Negative.	
Lot No. 275. 60 bugsinfected Oct. 15, 1921.	25 days. Positive.		48 hours; 60 bugs bit tail of mouse 71. Negative.	
Lot No. 276. 39 bugsinfected Oct. 15, 1921.	25 days. Positive.		48 hours; 39 bugs bit tail of mouse 72. Negative.	
Lot No. 283. 79 bugsinfected Oct. 21, 1921.	2 days. Positive.		48 hours; 79 bugs bit tail of mouse 73. Negative.	
Lot No. 284. 27 bugsinfected Oct. 21, 1921.	2 days. Positive.		48 hours; 27 bugs bit tail of mouse 74. Negative.	

V. TRANSMISSION OF TULARÆMIA BY THE MOUSE LOUSE POLYPLAX SERRATUS (BURM.).

By EDWARD FRANCIS, Surgeon, and G. C. LAKE, Passed Assistant Surgeon, United States Public Health Service.

In two experiments healthy white mice were placed in contact in glass aquarium jars with white mice which had been inoculated subcutaneously with diluted heart's blood of mice dead from tularæmia. Not only did the inoculated mice die from tularæmia but the healthy mice contracted the disease and died with typical lesions.

TRANSMISSION BY CONTACT.

One of the experiments just cited lasted 15 days, from June 24 to July 9.

On the first day of this experiment, 24 healthy mice and 2 mice inoculated two days previously were introduced into a glass aquarium jar 18 inches in diameter. As soon as an inoculated mouse died it was replaced by another inoculated mouse in the third day of the disease; thus the jar was kept constantly supplied throughout the experiment with two inoculated mice in the later stages of the disease. By the fifteenth day of the experiment all of the 24 healthy mice had died, 16 having died with the typical condition of the spleen due to *Bacterium tularense*. Nine of the 16 spleens were rubbed on the shaved abraded skin of 9 guinea pigs, all of which died with the typical lesions of tularæmia. Of the 16 mice referred to, 1 died on the fifth day of the experiment, 4 died on the seventh day, 3 on the eighth day, 1 on the ninth day, 1 on the tenth day, 1 on the eleventh day, 1 on the twelfth day, 2 on the thirteenth day, 1 on the fourteenth day, and 1 on the fifteenth day. Eight of the 24 mice died from causes other than tularæmia, 4 having died during the first three days of the experiment, and 4 having died later in the experiment.

In a similar experiment, 4 inoculated white mice and 26 healthy white mice were introduced into a glass aquarium jar on August 20, after which date no additional mice were added. The four inoculated mice died within the first five days, with typical lesions of tularæmia. On September 15 the last remaining healthy mouse died with typical lesions of the spleen, which was rubbed on the abraded skin of a guinea pig and caused its death on the sixth day from tularæmia. During the interval of 25 days between August 20 and September 15, the 26 healthy mice all died, the majority of them showing typical lesions of tularæmia.

After the death of the last mouse in the aquarium jar and its removal therefrom, the jar was left undisturbed for eight days. At the expiration of this time eight healthy mice were introduced into

the jar. They remained well, thus showing the absence of any residual infection.

Throughout these two contact experiments we searched the mice and the bedding for parasites and tested the infectivity of the urine of infected mice, and also noted whether dead infected mice had been mutilated by contacts, our object being to determine the mode of transmission in these experiments. The several possible factors concerned in the transmission will now be considered.

INFECTIVITY OF MOUSE URINE.

Throughout our experiments we kept going in white mice strain "S" of *Bacterium tularense* by subcutaneous inoculations of the heart's blood of a recently dead infected mouse into a healthy mouse. Urine voided naturally by living infected mice was collected between 72 and 96 hours after inoculation, since death quite regularly occurred by the end of 96 hours.

The urine collected from four mice was injected subcutaneously into 4 guinea pigs, respectively. The guinea pigs all died acutely with typical lesions of tularæmia. The amounts of urine injected were 10 gtts., 12 gtts., ½ c. c., and ½ c. c. The infectivity of similar samples of mouse urine was also tested by feeding the urine to white mice. The first mouse ate 12 gtts. of such urine mixed with corn meal. The second mouse ate ½ c. c. urine mixed with corn meal on six consecutive occasions spaced about a week apart, thus consuming about 3 c. c. Both mice remained well. We concluded that urine, though infected, was not an agent of transmission in nature.

CANNIBALISM AND INFECTION.

White mice mutilate and sometimes completely eat their dead comrades. While we made no observation on whether a healthy mouse will contract the infection by eating a mouse dead from tularæmia, we did feed six white mice with a small amount of the liver of a rabbit which had just died with typical lesions of tularæmia. All the mice died within five days: One died on the third day, two on the fourth, and three on the fifth day. In each instance the mouse's spleen was rubbed on the shaved, abraded skin of a guinea pig, causing acute death with typical lesions of tularæmia. In our contact experiments, infected mice, when found dead, occasionally showed evidence of mutilation by their comrades.

INFESTATION OF WHITE MICE WITH LICE AND MITES.

Examination of the mice revealed the presence of the blood-sucking louse *Polyplax serratus* (Burm), the blood-sucking mite *Liponyssus isabellinus*, and two species of non-blood-sucking mites. No other

parasites were found either on the mice or in the hay used for bedding.

The lice were present in variable numbers; some mice had none; two had about 60 each; 30 lice was considered a large number for a single mouse; about 15 were commonly found on a mouse. The mites were found in much smaller numbers; most mice had none; 20 was the largest number found on a mouse; 9 was the next largest number found; the number on a mouse hardly ever exceeded 4 or 5.

EXPERIMENTAL TRANSMISSION BY THE MOUSE LOUSE, POLYPLAX SERRATUS.

Experiments were planned to determine definitely the rôle of the mouse louse as an agent in the transfer of the infection from infected mice to healthy mice. Mice were inoculated subcutaneously with diluted blood taken from the heart of a mouse dead from tularæmia, and upon the death of the inoculated mouse his hair was pulled out, transferred to a sheet of white paper and examined for lice. The hair was well teased apart with needles, and any moving object was readily seen in the white hair on the white background. A glance at any moving object with a hand lens was sufficient to exclude the possibility of the parasite being other than a louse. The few hairs to which a louse was clinging were transferred to a clean petri dish, and thus the lice were collected into a pile of very few hairs. The small pile of hairs was then picked up with a forceps and transferred to beneath the hair of the back of a healthy white mouse which was being held. The lice almost instantly left the pile of hairs and disappeared among the hairs of the living mouse. Mice to which lice were thus transferred were placed separately in clean jars for observation.

The time which elapsed between the removal of lice from a dead mouse and their transfer to a healthy mouse probably never exceeded an hour. No effort was made to learn how long lice would remain infected. We did, however, remove from three mice three lots of lice, numbering about 25 to the lot, and kept them on hair in petri dishes at room temperature in August; they were all dead by the end of 48 hours. The time which elapsed between the death of a mouse and the removal of its lice was likewise not definitely determined, although it never exceeded 18 hours. An effort was made to remove the lice as soon as possible. Lice were collected in the morning from mice which died during the previous night, and lice were collected during the day from mice dying during the day.

These transmission experiments, as conducted, excluded the entrance of several possible factors which might have operated to make the contact experiments successful, namely, the agency of the blood-sucking mites, and the eating of infected mice, or possibly infected secretions or excretions, by healthy mice.

The strain of *Bacterium tularense* (S) used was one isolated from a human case in Utah in the summer of 1920.

First series.—In this series, transmission of tularæmia to healthy mice was effected by the transfer to them of lice removed from mice dead after subcutaneous inoculation with diluted heart's blood of infected mice. Eleven healthy mice were thus infested, the number of lice used for each mouse varying from 10 to 43. Nine of the 11 died with typical lesions of the spleen due to tularæmia, and, moreover, the spleen in each instance, when rubbed on the shaved, abraded skin of a guinea pig, caused the death of this animal with the typical lesions of the disease. Two mice of this series remained negative; 1 had been infested with 10 lice and the other with 33 lice.

Second series.—Transmission of tularæmia was effected in the second series by the transfer of lice from louse-infected mice of the first series to healthy mice. Six healthy mice were thus infested, the number of lice transferred to each mouse varying from 5 to 25. Three of the six mice died with typical lesions of the spleen due to tularæmia, and the spleen in each instance, when rubbed on the shaved, abraded skin of the abdomen of a guinea pig caused the death of this animal with the typical lesions of the disease. Three mice of this series remained well; they had been infested with 5, 9, and 20 lice, respectively.

INFECTIVITY OF THE MITE LIPONYSSUS ISABELLINUS.

Ten mites of the species *Liponyssus isabellinus* were collected while on the ends of the hairs about to leave the body of a white mouse dead from tularæmia after subcutaneous inoculation with infected blood. The mites were rubbed in a mortar with saline solution and the suspension was injected subcutaneously into a white mouse, causing its death in 4½ days, with typical lesions of tularæmia. A portion of the spleen of the latter mouse was used for subcutaneous injection of one guinea pig, while another portion was rubbed on the shaved abraded skin of another guinea pig; both guinea pigs died acutely with typical lesions of tularæmia. Had these blood-sucking mites been present in sufficient numbers, transmission experiments would have been conducted along the lines followed in the louse transmission.

FAILURE OF ATTEMPTS TO RENDER MICE LOUSE-FREE AND MITE-FREE.

We endeavored to render white mice free from parasites. Nicotine sulphate in water (1:1000), 95 per cent alcohol, undiluted kerosene, and undiluted gasoline were used. A lousy mouse dipped into either of these agents suffered considerable toxic effects; he was rendered apparently free from parasites for a few days, but if examined 7 to 10

days later, he was again lousy. These agents did not kill the eggs. We failed to find a delousing agent into which a mouse could be dipped 4 or 5 times at intervals of a week without causing death or injury to the mouse. Had we been able to render our mice lice-free and mite-free we would have conducted with them a contact experiment similar to our two contact experiments with the expectation that transmission to contacts would not occur.

THE ABSENCE OF LICE FROM HOUSE MICE.

An observation was made on the occurrence of lice on the ordinary house mouse found in various parts of the laboratory. The mice were caught during the night in snap traps and were collected each morning and examined. The hair of 56 mice were pulled out and searched for lice and mites. Only 1 louse and 16 mites were found. This louse was the rat louse *Polyplax spinulosa* (Burm). No specimen was found of the mouse louse *Polyplax serratus* (Burm) so commonly found on our white mice.

The apparent absence of lice from house mice has a bearing on the question of whether tularæmia would become epizoötic in house mice if by chance infected ones got at large.

The susceptibility of "gray" mice to the infection has already been shown by McCoy.[2] In an experiment of ours, house mouse No. 1, inoculated subcutaneously with the heart's blood of an infected white mouse, died with typical lesions of tularæmia. House mouse No. 2, inoculated with the heart's blood of No. 1, died with typical lesions of tularæmia. The infection was similarly carried over to No. 3. The spleen of No. 3, when rubbed on the shaved, abraded skin of a guinea pig caused its death with typical lesions of tularæmia.

SUMMARY.

The transmission of tularæmia was effected in 12 out of 17 attempts through the agency of the mouse louse (*Polyplax serratus*) by the transfer of lice from white mice dead of tularæmia to healthy white mice, the intervals elapsing between infestation of the healthy mice and their deaths varying from 5 to 12 days, the average being 7¼ days. The number of lice transferred in the 12 successful attempts varied from 12 to 43, the average being 25. The intervals which elapsed between the deaths of infected mice and the transfer of their lice to healthy mice varied from a few minutes to 18 hours. Transmission of tularæmia by lice was thus effected to two series of mice, the first series being infected by lice removed from inoculated mice and the second series being infected by lice removed from the louse-infected mice of the first series.

[2] A plaguelike disease of rodents. By George W. McCoy, Passed Assistant Surgeon, United States Public Health Service. Public Health Bulletin No. 43, April, 1911.

When inoculated mice were dropped into a jar in contact with lousy healthy mice, the infection killed off all the healthy mice in 25 days. Transmission in this case was probably due to lice.

Blood-sucking mites of the species *Liponyssus isabellinus* removed from an infected white mouse were crushed and injected subcutaneously into another white mouse causing its death from tularæmia.

The urine of infected white mice was infective for guinea pigs when injected subcutaneously into the latter. Similar urine failed to infect white mice when fed to them on corn meal.

The mouse louse *Polyplax serratus* commonly found on our white mice was absent from 56 house mice caught in snap traps in the laboratory.

Acknowledgment.—The determinations of specimens of lice and mites were made by Dr. H. E. Ewing, of the Bureau of Entomology, Department of Agriculture.

Transmission of tularæmia in white mice by the mouse louse Polyplax serratus.

Series.	Infected mice from which lice were removed.			Healthy mouse to which lice were transferred.	Length of time between infestation with lice and death of mouse.	Results: "Positive" means death of a guinea pig with typical lesions of tularæmia, the guinea pig having been rubbed on the shaved abraded skin of the abdomen with the spleen of the louse-infected mouse.
	No. of mouse.	Date of death.	Number of lice transferred from infected mouse to healthy mouse.		*Days.*	
First series: Transmission of tularæmia by lice, transferred from inoculated mice to healthy mice.	SM156	July 2, 1921	15	Mouse No. 1	5	Positive.
	SM158	do	14	do		
	SM155	July 3, 1921	3	Mouse No. 2	9	Do.
	SM160	do	5	do		
	SM161	do	15	do		
	SM162	do	10	do		
	SM163	July 4, 1921	15	Mouse No. 3	9	Do.
	SM164	do	12	do		
	SM165	do	15	Mouse No. 4	6	Do.
	SM166	do	8	do		
	DC6	do	6	do		
	DC2	July 5, 1921	30	Mouse No. 5	7	Do.
	DC2	do	30	Mouse No. 6	9	Do.
	SM172	July 6, 1921	30	Mouse No. 7	5	Do.
	SM173	do	13	do		
	SM176	July 7, 1921	15	Mouse No. 8	0	Do.
	SM178	do	12	Mouse No. 9	12	Do.
	SM181	July 9, 1921	20	Mouse No. 10		Negative.
	SM185	do	13	do		
	DC11	July 10, 1921	10	Mouse No. 11		Do.
Second series: Transmission of tularæmia was effected by lice transferred from louse-infected mice of first series to healthy mice.	1	July 7, 1921	14	Mouse No. 20	5	Positive.
	7	July 11, 1921	25	Mouse No. 21	7	Do.
	5	July 12, 1921	20	Mouse No. 22		Negative.
	2	do	5	Mouse No. 23		Do.
	8	July 13, 1921	12	Mouse No. 24	7	Positive.
	9	July 19, 1921	9	Mouse No. 25		Negative.

VI. CULTIVATION OF BACTERIUM TULARENSE ON MEDIUMS NEW TO THIS ORGANISM.

By EDWARD FRANCIS, Surgeon, United States Public Health Service.

The only culture mediums reported heretofore for the cultivation of *Bacterium tularense* are coagulated hen's egg yolk, originally used by McCoy and Chapin,[3] and hen's ovomucoid with a trace of yolk, recommended by Wherry and Lamb.[4] All attempts at cultivation on other laboratory mediums had failed.

The writer now reports the cultivation of this organism on (1) serum glucose agar, (2) glucose blood agar, (3) blood agar, (4) each of the foregoing mediums plus a piece of fresh, sterile rabbit spleen.

It is a common practice in laboratories to adapt an organism to growth on ordinary mediums after original isolation from man or animals has been accomplished by cultivation on some special medium. The mediums which are here reported for *Bacterium tularense* were used for original isolations of this organism from animals, and therefore the question of acquired adaptability to a new medium brought about by previous cultivation on a special medium is not involved.

The strains used for this cultural work were three human strains from Utah and one ground-squirrel strain from California, all obtained in 1920. These strains were originally obtained by the inoculation of human or squirrel tissue into guinea pigs, and they were subsequently passed for many generations through guinea pigs or rabbits, always by subinoculation of infected tissues. Original isolation from animals of pure cultures of these strains was accomplished by use of the proposed mediums; the resultant cultures have never been on egg medium either before or since their first isolation.

COMPOSITION OF MEDIUMS.

(1) *Serum glucose agar.*—Beef infusion containing 1 per cent peptone and 1½ per cent agar adjusted to a reaction having a p_H of 7.6 is kept on hand in stock. When needed, the stock agar is melted and brought to 45° C. in a water bath, at which temperature there is added 1 per cent glucose from a sterile 50 per cent solution of glucose and 5 per cent sterile horse serum. This is immediately tubed, slanted, and incubated 48 hours to insure sterility.

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[3] Bacterium tularense, the Cause of a Plague-like Disease of Rodents. By George W. McCoy and Charles W. Chapin, Passed Assistant Surgeons, United States Public Health Service. Public Health Bulletin No. 53, January, 1912.

Further Observations on a Plague-like Disease of Rodents with a Preliminary Note on the Causative Agent, Bacterium tularense. By George W. McCoy and Charles W. Chapin, Passed Assistant Surgeons, United States Public Health Service. The Journal of Infectious Diseases, Vol. X, No. 1, January, 1912, pp. 61-72.

[4] Infection of Man with Bacterium tularense. By William B. Wherry and B. H. Lamb. Journal of Infectious Diseases, 1914, vol. 15, p. 331.

(2) *Glucose blood agar.*—This is the same as (1) except that 5 per cent defibrinated rabbit blood is substituted for the horse serum.

(3) *Blood agar.*—This is the same as (2) except that no glucose is added.

(4) *Mediums (1), (2), and (3) plus spleen tissue.*—A spleen is removed from a healthy rabbit and under sterile precautions is cut into pieces of about 3 mm. diameter. One piece is rubbed on the slanted surface of each tube of a portion of mediums (1), (2), and (3) and the piece of spleen is left remaining on the surface of each slant just above the water of condensation. After 48 hours' incubation the tubes, if sterile, are ready for inoculation.

COAGULATED HEN'S EGG YOLK.

At this place it will be well to give also the composition of the coagulated hen's egg yolk described by McCoy and Chapin. Fresh eggs are scrubbed with a brush in soap and water, if fecal matter is present on the shells, and then placed in a wire basket. The basket containing the eggs is dipped into 95 per cent alcohol for a few seconds, after which time it is withdrawn and the small amount of alcohol which still remains on the basket and eggs is ignited in order to remove the alcohol and help sterilize the shells.

While one person with clean hands holds an egg, grasping it at each end, an assistant strikes the shell in its middle with a sterile knife with sufficient force to crack the shell. The whites are separated from the yolks by decanting from one half of the shell to the other, thus allowing the whites to drain away while the yolks are saved and collected in a sterile beaker.

The volume of yolks is measured in a sterile graduate and to this is added sterile normal saline solution in the proportion of 40 per cent saline solution to 60 per cent egg yolk. Mix thoroughly. Tube in sterile test tubes, using a sterile funnel.

Place the tubes in metal racks constructed so as to allow one-half-inch space between the tubes for circulation. Heat the racked tube, in a slanting position for the first half hour at 70° C., and for the second half hour at 72° C. A uniform temperature is best maintained for this purpose in a water-jacketed chamber. The chamber should contain about a half inch of water above which the racks of tubes are exposed in the moist, heated air. After coagulation, paraffined sterile cork stoppers are substituted for the cotton plugs and the tubes are incubated upright for three or four days to insure against a slow-growing contamination.

Instead of the jacketed chamber one may, with patience, use an Arnold steam sterilizer, a board having been placed at the bottom to protect the tubes from the direct steam.

The finished medium should be soft; that is, the surface of the slant should yield slightly when pressed with a platinum loop, and to that end the medium should not be overheated. A glazed surface results from overheating. The tubes should be stored in the cold room, unexposed to the light. The water of condensation in a batch of medium which grew the organism very well showed a reaction having a p_H of 6.8. No titration or adjustment of reaction has been done on batches of this medium used for routine cultivation of the organism in the laboratory.

CULTIVATION ON SERUM GLUCOSE AGAR.

Serum glucose agar was successfully used (1) for original isolation of strains from the spleens of infected rabbits, (2) for second and third isolations of strains from the spleens of infected guinea pigs, (3) for subcultures (without the addition of a piece of fresh, sterile rabbit spleen), and (4) for subcultures (with the addition of a piece of fresh, sterile rabbit spleen).

(1) *Original isolation of strains.*—Human strains "J" and "G" and ground-squirrel strain "S F" were isolated on serum glucose agar by planting a piece of infected rabbit spleen on serum glucose agar in each instance. (See Table I, and Table III, animals 1, 3, and 4.) These human strains had been carried over from July 3 and September 9, 1920 (the dates on which they left the humans), to April 11, 1921 (the date on which they were cultured), in laboratory animals, i. e., guinea pigs and rabbits. These human strains having been carried over exclusively by animal passages for 9 and 7 months, respectively, original isolations were made on serum glucose agar. The ground-squirrel strain had been carried over from May, 1920 (the date on which it left a California ground squirrel), to April 10, 1921 (the date on which it was cultured), in laboratory animals, guinea pigs, and rabbits. This California ground-squirrel strain having been carried over exclusively by animal passages for 11 months, original isolation was made on serum glucose agar.

On April 10 and 11, 1921, each of the three strains was inoculated on a serum glucose agar slant by transferring to the surface of the medium a piece about 3 mm. in diameter, taken under sterile precautions, from the spleen of a rabbit dead from tularæmia; the piece of spleen was rubbed over the surface of the medium as forcibly as the consistency of the latter would permit and then left to remain on the solid medium just above the water of condensation. The inoculated tubes were placed at 37° C. for eight days, at the end of which time they were observed for the first time and found to have a growth which was not examined microscopically, but the tubes were replaced in the incubator under the impression that the growths

were contaminations. The tubes remained at 37° for a month longer without observation, at the end of which time they were given another examination preliminary to discarding. The presence of the growth saved the tubes from the discard and they remained another month at room temperature, when, on June 23 and August 23, the growths were examined microscopically and found to simulate *Bacterium tularense*.

Confirmation of these original cultures was obtained when subcultures in the fifth generation on glucose blood agar plus a piece of fresh, sterile rabbit spleen caused acute death with typical lesions of tularæmia in a set of guinea pigs which had been rubbed on the shaved abraded skin of the abdomen with those subcultures.

Further confirmation was obtained when cultures derived from the spleens of the above set of guinea pigs caused acute death with typical lesions of tularæmia in a second set of guinea pigs, the second set having been rubbed on the shaved abraded skin of the abdomen with subcultures in the fifth generation on glucose blood agar plus a piece of fresh sterile rabbit spleen. (For details see Table I.)

(2) *Second and third isolations.*—The two sets of guinea pigs just referred to not only gave confirmation to the identity of the original cultures which were isolated on serum glucose agar but afforded opportunities for the second and third isolations of those strains from animals on serum glucose agar as follows: On the death of a guinea pig of either set a piece of its spleen was planted on a tube of serum glucose agar and incubated at 37° C. Ten tubes were thus planted from 10 guinea pigs. (See Table III, animals 5 to 15.) Five tubes showed growth and five showed no growth.

The five growths therefore constituted second and third isolations of our three human strains and one ground squirrel strain on serum glucose agar.

(3) *Subcultivation on serum glucose agar.*—Plain serum glucose agar was used for subcultures as follows: Starting with a piece of infected spleen of a rabbit or guinea pig, this was planted on a tube of serum glucose agar and the resultant growth was transferred for its second generation to a tube of serum glucose agar. Thirteen serum glucose agar tubes were thus inoculated with six cultures having an antecedent history as indicated. Four of the tubes developed growth after an average of six days and nine failed to grow. (See Table III, footnotes 4, 5, and 6.) The four growths constituted subcultivation on serum glucose agar.

(4) *Subcultivation on serum glucose agar* (plus a piece of fresh sterile rabbit spleen).—Subcultivation from serum glucose agar to serum glucose agar plus a piece of fresh sterile rabbit spleen was successful in 14 out of 27 attempts; the average time which elapsed before the appearance of growth on the 14 tubes was 2½ days.

Three cultures were thus carried for the third to sixth generations on serum glucose agar plus a piece of fresh sterile rabbit spleen, the first generation in each case having been obtained on a tube of serum glucose agar inoculated with a piece of spleen of an infected guinea pig. (See Table III, animals 5, 6, and 12.) The fifth generation of culture 1 was injected subcutaneously into a guinea pig, causing its death in three days with typical lesions of tularæmia. The sixth generation of culture 1 was rubbed on the shaved abraded skin of a guinea pig and caused its death on the twelfth day with typical lesions of tularæmia. (See Table I, footnote 2.) The sixth generations of cultures 2 and 3 when injected subcutaneously each failed to kill a guinea pig.

Summary.—Serum glucose agar *per se* is a poor medium for the cultivation of *Bacterium tularense* because subcultures from serum glucose agar to serum glucose agar grew in only 30 per cent of the instances. Serum glucose agar is a fair medium for the cultivation of *Bacterium tularense* if the surface of the medium is provided with a piece of fresh spleen tissue. Such tissue may be supplied in either of two ways.

If the inoculating material is in substance a piece of fresh tissue, as is the case when a piece of the spleen of an infected rabbit or guinea pig is planted on a serum glucose agar tube, growth may be expected in 48 per cent of the tubes. Growth did occur in 11 out of 23 such attempts, the average time before the appearance of growth being 8½ days.

If the inoculating material is a culture, as is the case in subcultivation, and a piece of fresh sterile rabbit spleen has been supplied to the surface of the serum glucose agar tubes, growth may be expected in 51 per cent of the tubes. Growth did occur in 14 out of 27 such attempts, the average time which elapsed before the appearance of growth on the 14 tubes being 2½ days.

It was noted, however, that the growth of *Bacterium tularense* on serum glucose agar under all conditions tends to become scanty and its virulence tends to become either diminished or lost.

CULTIVATION ON GLUCOSE BLOOD AGAR.

Glucose blood agar was successfully used (1) for original isolation of strains from the spleens of infected rabbits, (2) for second and third isolations of strains from the spleens, liver, and heart's blood of infected guinea pigs, (3) for subcultures (without the addition of a piece of fresh sterile rabbit spleen), and (4) for subcultures (with the addition of a piece of fresh sterile rabbit spleen).

(1) *Original isolation of strains.*—Human strains "J" and "S" were isolated on glucose blood agar by planting a piece of infected

rabbit spleen on a glucose blood agar tube in each case. (See Table III, animals 1 and 2.)

These human strains had been maintained in the laboratory in guinea pigs and rabbits from September 9 and July 3, 1920 (the dates on which they left the humans), to April 11 and 13, 1921, the dates on which they were first cultured; they had not been on any culture medium during that period. On April 11 and 13 these strains were inoculated on glucose blood agar slants by transferring to the surface of the medium a piece about 3 mm. in diameter taken under sterile precautions from the spleen of a rabbit dead from tularæmia. The tubes were incubated at 37° and not observed for 8 days, at the end of which time growth was present in case of the "J" strain, but no notation was then made of growth of "S" strain, although it was noted at a later date. As in the case of original cultures on serum glucose agar these cultures remained unobserved in the incubator for one month longer, when, on May 24, strain "J" was subcultured and it was subcultured next again on June 22; whereas strain "S" went from April 13 to July 4 before the first subculture was made.

Confirmation of the original cultures was obtained when subcultures in the fifth generation on glucose blood agar, plus a piece of fresh, sterile rabbit spleen caused acute death with typical lesions of tularæmia in a set of guinea pigs which had been rubbed on the shaven, abraded skin of the abdomen with those subcultures.

Further confirmation was obtained when cultures derived from the spleens of the above set of guinea pigs caused acute death with typical lesions of tularæmia in a second set of guinea pigs, the second set having been rubbed on the shaved, abraided skin of the abdomen with subculture in the fifth generation on glucose blood agar, plus a piece of fresh, sterile rabbit spleen. (For details see Table II.)

(2) *Second and third isolations.*—The two sets of guinea pigs just referred to not only gave confirmation to the identity of the original cultures which were isolated on glucose blood agar, but afforded opportunity for the second and third isolations of these strains from animals on glucose blood agar as follows: On the death of a guinea pig of either set, a piece of its spleen was planted on a tube of glucose blood agar and incubated at 37° C. Thirteen tubes were thus planted from 13 guinea pigs, including liver and heart's blood in two instances. (See Table III, animals 5 to 15.) All of the 13 tubes showed growth after an average of a little less than four days.

These 13 growths therefore constituted second and third isolations of these two human strains on glucose blood agar.

(3) *Subcultivation on glucose blood agar.*—Plain glucose blood agar was used for subculture as follows: Starting with a piece of infected spleen of a rabbit or guinea pig, this was planted on a tube of glucose

blood agar and the resultant growth was transferred for its second generation to a tube of glucose blood agar. Five glucose blood agar tubes were inoculated with five cultures having an antecedent history as indicated. None of the tubes developed any growth. (See Table III, animals 21 to 25.)

Four cultures grown for two or three generations on glucose blood agar, plus a piece of fresh sterile rabbit spleen, were subsequently transferred for two or three generations to glucose blood agar; the transfer was accompanied by a falling off in the abundance of growth. Moreover, a falling off in virulence also took place because the last generation of each culture was rubbed on the shaven, abraded skin of one or two guinea pigs, with the result that one guinea pig died acutely on the seventh day, two died tardily on the twelfth day, one died subacutely on the twenty-third day, and two guinea pigs, vaccinated with the fourth culture, remained well. (See Table I, footnote 1, and Table II, footnotes 1, 2, and 3.)

(4) *Subcultivation on glucose blood agar* (plus a piece of fresh, sterile rabbit spleen).—Fifteen cultures which had been isolated by planting a piece of the spleen of an infected rabbit or guinea pig on either glucose blood agar or serum glucose agar, with or without the addition of a piece of fresh, sterile rabbit spleen were subcultured from the second to the fifth generations on glucose blood agar plus a piece of fresh sterile rabbit spleen; the fifth generation in each instance was rubbed on the shaven abraded skin of a guinea pig causing its death acutely with typical lesions of tularæmia. (See Tables I and II, and Table III, footnote 5.)

This medium was successful in 88 out of 103 attempts at subcultivation of the organism; the average time which elapsed before the appearance of growth on the 88 tubes was two days; growth appeared before the end of 24 hours in 61 of the 88 tubes.

Isolation of cultures from animal tissues to glucose blood agar plus a piece of fresh sterile rabbit spleen was successful in 21 out of 27 attempts. The animal tissues consisted of a piece of the spleen of 19 infected rabbits and guinea pigs, a piece of the liver of one infected guinea pig, the heart's blood of two infected guinea pigs, and the heart's blood of five infected white mice. The average of time before the appearance of growth on the 21 tubes was five days. (See Table III.)

Summary.—Glucose blood agar *per se* is not a good medium for the cultivation of *Bacterium tularense*. The plain glucose blood agar is a good medium only when the infected material with which it is inoculated is in substance a piece of fresh tissue as represented by a piece of the infected spleen of a rabbit or guinea pig. This was the case when 25 rabbits or guinea pigs dead from tularæmia each furnished a piece of spleen which was inoculated on a tube of glucose

blood agar. Growth appeared after an average of 4⅓ days on 21 tubes; no growth appeared on 4.

Subcultures were made on 56 tubes of glucose blood agar; growth appeared after an average of three days on 26 tubes and failed to appear on 30. The growth, when subcultured on plain glucose blood agar became scanty and of lowered virulence.

The falling off in growth and virulence which accompanied the transfer of cultures to plain glucose blood agar is accounted for by the absence of a piece of fresh sterile rabbit spleen from the medium.

On the other hand, glucose blood agar plus a piece of fresh sterile rabbit spleen is a good medium, both for the isolation of *Bacterium tularense* from animals and for subcultivation. This medium was successful in 21 out of 26 attempts at isolation of the organism from the tissues of 26 animals. This medium was successful in 88 out of 103 attempts at subcultivation of the organism. Fifteen subcultures each in the fifth generation on this medium were rubbed on the shaven abraded skin of 15 guinea pigs, causing acute death in each instance with typical lesions of tularæmia, thus indicating no loss of virulence after subcultivation on this medium.

CULTIVATION ON BLOOD AGAR.

Blood agar was used (1) for second and third isolation of strains from rabbits and guinea pigs and (2) for subcultures.

(1) *Second and third isolations.*—No attempt was made at original isolation of our strains from animals on blood agar, but second and third isolations of these strains from animals were accomplished on blood agar. (See Table III, animals 5, 8, 10, 11, and 13.)

The organism was isolated on a blood agar slant which had been inoculated with a piece of the spleen of animal No. 5 dead after vaccination with the fifth generation of a culture which was *originally isolated* on serum glucose agar and subsequently grown for four generations on glucose blood agar plus a piece of fresh sterile rabbit spleen. Practically the same statement can be made concerning blood agar cultures obtained from animals 8, 10, 11, and 13.

Isolation on blood agar slants was also accomplished from the spleens of animals Nos. 21, 22, 23, 24, and 25 (see Table III), all of which had been inoculated with the heart's blood of Mouse 206. For one year previous to Mouse 206 the strain had had a continuous passage through mice, tame rabbits, and guinea pigs, back to human case "S" in Utah except that once during that year (from May 27 to June 6, 1921) the strain was carried on coagulated egg yolk for ten days.

(2) *Subcultivation.*—Subcultures on blood agar failed to grow in 13 out of 15 attempts; growth appeared on two tubes after three and six days, respectively.

Summary.—For the isolation of *Bacterium tularense* from infected animals, this medium was successful in 9 out of 15 attempts; growth appeared on the nine tubes after an average of seven days. The success of this medium for isolation from infected animals was undoubtedly due to the transfer to the medium of a piece of fresh tissue as represented by the piece of infected spleen of rabbit or guinea pig with which the tubes were inoculated. For subcultivation of this organism, blood agar *per se* is a poor medium, growth having been obtained only two times out of 15 attempts at subcultivation on this medium.

CONCLUSION.

The view heretofore held that *Bacterium tularense* will grow only on a culture medium containing egg yolk is no longer tenable.

The present paper contains reports of the growth of this organism in subcultures on serum glucose agar, glucose blood agar and blood agar. Growth on the above mediums *per se* is, however, scanty and of lowered virulence.

But these mediums take on an exalted value for the cultivation of this organism when supplied with a piece of fresh tissue; this tissue may be supplied either by the piece of spleen of the infected rabbit or guinea pig with which the medium is inoculated or a piece of fresh sterile spleen of a rabbit may be transferred to the medium, thereby preparing it to grow a subculture with which it may subsequently be inoculated.

The success of the cultural experiments here reported can not be ascribed to adaptation from a special medium to an ordinary medium because our mediums were employed for original isolations of the strains. The work here reported is with strains of *Bacterium tularense* which have never been on egg medium either before or since isolation; the only exception to this statement is contained in the very limited work done on animals 16 to 29 of Table III, in which the strain had had a few days' cultivation on coagulated egg yolk as exemplified by the statement at the bottom of page 109.

From the data presented in Table III there appears to be very little difference between the efficiency of glucose blood agar plus a piece of fresh rabbit spleen, and coagulated egg yolk. I am however of the opinion that coagulated egg yolk, carefully prepared, is still the best medium for routine isolation and cultivation of *Bacterium tularense*.

TABLE I.—*Serum glucose agar used for original isolation of human strains "J" and "G" and squirrel strain "SF" of Bacterium tularense. Subcultures made on glucose blood agar and serum glucose agar, each being supplemented by a piece of fresh sterile rabbit spleen.*

Human strain "J." (See Table III, animal No. 1.)	Human strain "G." (See Table III, animal No. 3.)	California ground squirrel strain "SF." (See Table III, animal No. 4.)
1921.	1921.	1921.
Apr. 11. Piece of infected rabbit spleen planted on serum glucose agar.	Apr. 11. Piece of infected rabbit spleen planted on serum glucose agar.	Apr. 10. Piece of infected rabbit spleen planted on serum glucose agar.
June 23. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.	June 22. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.[1]	Aug. 23. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.
July 4. Subcultured on above medium.	July 4. Subcultured on above medium.	Aug. 25. Subcultured on above medium.
July 9. Subcultured on above medium.	July 10. Subcultured on above medium.	Aug. 26. Subcultured on above medium.
July 14. Subcultured, fifth generation.	July 14. Subcultured, fifth generation.	Aug. 28. Subcultured, fifth generation.
July 18. Vaccinated a guinea pig with a loop of 4-day culture of the fifth generation.	July 18. Vaccinated guinea pig with a loop of a 4-day culture of the fifth generation.	Aug. 29. Subcultured, sixth generation.
July 25. Guinea pig dying, chloroformed, planted pieces of its spleen on 5 mediums. (See Table III, animal No. 7.)	July 25. Guinea pig dead, planted pieces of its spleen on 6 media. (See Table III, animal No. 12.)[2]	Aug. 31. Subcultured, on above medium.
Aug. 21. Subcultured from glucose blood agar to glucose blood agar plus a piece of fresh sterile rabbit spleen.	Aug. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium.	Sept. 6. Subcultured on above medium.
Aug. 31. Subcultured on above medium.	Aug. 21. Subcultured to same medium.	Sept. 14. Subcultured on above medium.
Sept. 2. Subcultured on above medium.	Aug. 23. Subcultured to same medium.	Sept. 2. Vaccinated one guinea pig with a loop of a 4-day culture of the sixth generation.
Sept. 3. Subcultured, fifth generation.	Aug. 24. Subcultured, fifth generation.	Sept. 5. Injected another guinea pig subcutaneously with a loop of an 8-day culture of the fifth generation.
Sept. 5. Vaccinated a guinea pig with a loop of 48-hour culture of the fifth generation.	Aug. 26. Vaccinated a guinea pig with a loop of 48-hour culture of the fifth generation.	Sept. 12. Both guinea pigs dead with typical lesions of tularæmia. Vaccinated a guinea pig with the spleen of the guinea pig which had been injected subcutaneously.
Sept. 11. Guinea pig died with lesions of the lymph glands, spleen and liver typical of tularæmia.	Sept. 1. Guinea pig dead with typical lesions of tularæmia.	Sept. 19. Guinea pig died with typical lesions of tularæmia.

[1] This culture of June 22 being in the second generation was also subcultured for the third, fourth, and fifth generations on glucose blood agar without the addition of a piece of fresh sterile rabbit spleen. The fifth generation was then vaccinated on two guinea pigs on July 23. Both guinea pigs were dead Aug. 4 with typical lesions of tularæmia.

[2] On July 25 one piece of spleen was planted on serum glucose agar.

Aug. 21. Subcultured on above medium.
Sept. 1. Subcultured on serum glucose agar plus a piece of fresh sterile rabbit spleen.
Sept. 9. Subcultured on above medium.
Sept. 11. Subcultured, fifth generation.
Sept. 14. Subcultured, sixth generation.
Sept. 14. Guinea pig injected subcutaneously with 3-day culture of the fifth generation.
Sept. 17. Guinea pig dead with typical lesions of tularæmia.
Sept. 18. Vaccinated a guinea pig with a loop of 4-day culture of the sixth generation.
Sept. 30. Guinea pig died with typical lesions of tularæmia.

TABLE II. *Glucose blood agar used for original isolation of human strains "J" and "S" of Bacterium tularense. Subcultures on glucose blood agar plus a piece of fresh sterile rabbit spleen.*

Human strain "J." (See Table III, animal No. 1.)	Human strain "J." (See Table III, animal No. 1.)	Human strain "S." (See Table III, animal No. 2.)
1921.	1921.	1921.
Apr. 11. Piece of infected rabbit spleen planted on glucose blood agar.	Apr. 11. Piece of infected rabbit spleen planted on glucose blood agar.	Apr. 13. Piece of infected rabbit spleen planted on glucose blood agar.
May 24. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.	June 22. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.	July 4. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.
June 22. Subcultured on above medium.	June 24. Subcultured on above medium.[2]	July 10. Subcultured on above medium.[3]
July 10. Subcultured on above medium.[1]	July 3. Subcultured on above medium.	July 14. Subcultured on above medium.
July 14. Subcultured fifth generation.	July 9. Subcultured on above medium.	July 18. Vaccinated a guinea pig with a loop of four-day culture of the fourth generation.
July 18. Vaccinated a guinea pig with a loop of a 4-day culture of the fifth generation.	July 14. Subcultured sixth generation.	July 25. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table III, animal No. 8.)
July 27. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table III, animal No. 5.)	July 18. Vaccinated a guinea pig with a loop of a 4-day culture of the sixth generation.	Aug. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium.
Aug. 8. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium.	July 31. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table III, animal No. 6.)	Aug. 21. Subcultured to same medium.
Aug. 14. Subcultured to same medium.	Aug. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium.	Aug. 23. Subcultured to same medium.
Aug. 21. Subcultured to same medium.	Aug. 21. Subcultured to same medium.	Aug. 24. Subcultured fifth generation.
Aug. 23. Subcultured to same medium.	Aug. 23. Subcultured to same medium.	Aug. 26. Vaccinated a guinea pig with a loop of 48-hour culture of the fifth generation.
Aug. 24. Subcultured sixth generation.	Aug. 24. Subcultured fifth generation.	Sept. 1. Guinea pig dying chloroformed, planted pieces of its spleen on 7 mediums. (See Table III, animal No. 9.)
Aug. 26. Vaccinated a guinea pig with a loop of a 48-hour culture of the sixth generation.	Aug. 26. Vaccinated a guinea pig with a loop of a 48-hour culture of the fifth generation.	Sept. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium.
Sept. 9. Guinea pig dying, chloroformed, showed typical lesions of tularæmia.	Sept. 9. Guinea pig dying, chloroformed, showed typical lesions of tularæmia.	

[1] This subculture planted July 10 being in the fourth generation was also subcultured for the fifth and sixth generations on glucose blood agar without the addition of a picee of fresh sterile rabbit spleen. This sixth generation was on July 23 vaccinated on a guinea pig which on Aug. 15 was dying and was chloroformed; it showed the typical lesions of the subacute type of tularæmia.

[2] This subculture planted June 24 being in the third generation was also subcultured for the fourth, fifth, and sixth generations on glucose blood agar without the addition of a piece of fresh sterile rabbit spleen. This sixth generation was on July 23 vaccinated on two guinea pigs which remained well.

[3] July 14. The subculture planted July 10 being in the third generation was also subcultured on July 14 on glucose blood agar without the addition of a piece of fresh sterile rabbit spleen.
July 17. Subcultured on above medium.
July 23. Vaccinated a guinea pig with a loop of 6-day culture of the fifth generation.
July 30. Guinea pig dying, chloroformed, planted pieces of its spleen on 8 mediums. (See Table III, animal No. 10.)
Aug. 7. Subcultured from blood agar to blood agar.
Aug. 21. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.
Aug. 23. Subcultured on the above medium.
Aug. 24. Subcultured fifth generation.
Aug. 26. Vaccinated a guinea pig with a loop of 48-hour culture of fifth generation.
Sept. 1. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table III, animal No. 11.)

TABLE III.—*Comparative value of several mediums for the isolation of Bacterium tularense from the spleens and heart's blood of 29 infected animals.*

No. of animal.	Either a piece of the spleen or the heart's blood of infected animals was planted.	Date planted.	Mediums on which pieces of infected tissue were planted.						
			Coagulated egg yolk.	Glucose blood agar slant plus a piece of fresh sterile rabbit spleen.	Glucose blood agar slant.	Blood agar slant.	Serum glucose agar slant.	Plain agar slant.	Glucose fermentation tube.
	(A) ORIGINAL ISOLATIONS OF BACTERIUM TULARENSE.	1921.							
1	Human strain "J" from spleen of rabbit J45R	Apr. 11	Good growth.		Growth after 8 days.[1]		Growth after 8 days.[1]	No growth.	No growth.
2	Human strain "S" from spleen of rabbit S51R	Apr. 13	Good growth after 48 hours.		Growth [1]		No growth	do	Do.
3	Human strain "G" from spleen of rabbit GCR3	Apr. 11	Growth		No growth		Growth after 8 days.[1]	do	Do.
4	California ground squirrel strain "SF" from spleen of rabbit SF25R.	Apr. 10	Growth, second day.		do		Growth after 9 days.[1]	do	Do.
	(B) SECOND AND THIRD ISOLATIONS OF ABOVE STRAINS.								
5	Spleen of guinea pig dead after vaccination[2] with subculture from animal No. 1.	July 27	Growth, third day.	About 100 colonies, fifth day.[1]	About 50 colonies, fourth day.[3]	5 colonies, fifth day.[4]	Growth, fifth day.[5]	No growth.	No growth.
6	do	July 31	Growth at end of 24 hours.	About 100 colonies, fourth day.[1]	do.[3]		3 colonies, fourth day.[5]	do	Do.
7	do	July 25	Contaminated.	Contaminated.	Pure culture, fifth day.[1]	Contaminated.	Contaminated.		
8	Spleen of guinea pig dead after vaccination[2] with subculture from animal No. 2.	do	Growth.	Good growth, fifth day.[1]	Good growth, fifth day.[6]	Growth, fifth day.[6]	No growth	No growth.	Do.
9	Spleen of guinea pig dead after vaccination[2] with subculture from animal No. 8.	Sept. 1	No growth	Good growth, third day.[4]	Good growth, third day.	No growth	do	do	Do.
10	Spleen of guinea pig dead after vaccination[2] with subculture from animal No. 2.	July 30	do	do.[1]	do.[4]	Good growth, third day.[4]	do	do	Do.

[1] Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen from second to fifth generations; the fifth generation was rubbed on the shaved abraded skin of a guinea pig causing its death acutely with typical lesions of tularæmia.

[2] "Vaccination" means that the shaved abraded skin was rubbed with a culture or with a piece of infected tissue.

[3] Subcultures on this same medium for the second, third, and fourth generations grew well.

[4] A subculture on this same medium for the second generation showed growth.

[5] Subcultured on serum glucose agar for the second and third generations and on serum glucose agar plus a piece of fresh sterile rabbit spleen for the fourth to seventh generations; the sixth and seventh generations injected subcutaneously failed to kill a guinea pig.

[6] A subculture on this same medium for the second generation failed to grow.

TABLE III.—*Comparative value of several mediums for the isolation of Bacterium tularense from the spleens and heart's blood of 19 infected animals*—Continued.

No. of animal.	Either a piece of the spleen or the heart's blood of infected animals was planted.	Date planted.	Mediums on which pieces of infected tissue were planted.						
			Coagulated egg yolk.	Glucose blood agar slant plus a piece of fresh sterile rabbit spleen.	Glucose blood agar slant.	Blood agar slant.	Serum glucose agar slant.	Plain agar slant.	Glucose fermentation tube.
	(B) SECOND AND THIRD ISOLATIONS OF ABOVE STRAINS—Continued.								
11	Spleen of guinea pig dead after vaccination[2] with subculture from animal No. 10.	Sept. 1	Good growth, third day.	Good growth, third day.	Good growth, third day.	1 dozen colonies, ninth day.	1 dozen colonies, ninth day.	No growth.	No growth.
	Heart's blood of guinea pig No. 11	do	No growth	25 colonies, third day.				do	Do.
12	Spleen of guinea pig dead after vaccination with subculture from animal No. 3.	July 25	Growth	Good growth, fifth day.[1]	Good growth, fifth day.[3]		30 colonies after 21 days.[7]	do	Do.
13	Spleen of guina pig dead after vaccination with spleen of guinea pig dead after subcutaneous injection with subculture from animal No. 4.	Sept. 19	Growth at end of 24 hours.	Growth at third 48 hours.	Growth at end of 48 hours.	Growth at end of 5 days.	Growth at end of 4 days.	Contaminated.	Do.
14	Spleen of guinea pig dead after subcutaneous injection with cultures grown on glucose blood agar.	July 28	No growth	1 dozen colonies, fifth day.[1]	50 colonies, third day.[4]	No growth	No growth	No growth.	Do.
	Heart's blood of guinea pig No. 14	do	do	Growth around tissue, ninth day.[1]	Growth, seventh day.[3]	do	do	do	Do.
15	Spleen of guinea pig dead after subcutaneous injection with cultures grown on glucose blood agar plus a piece of fresh sterile rabbit spleen.	July 24	Growth, second day.	Good growth, second day.[2]	Good growth, second day.[9]			do	
	Liver of guinea pig No. 15	do	do	do.[1]	do.[4]			do	
	(C) ISOLATIONS FROM VARIOUS SOURCES.								
16	Heart's blood of white mouse SM206 inoculated subcutaneously with heart's blood of mouse.	July 26	No growth	Growth, sixth day.[3]				No growth.	
17	Heart's blood of white mouse SM207 inoculated subcutaneously with heart's blood of mouse.	do	do	Growth eighth day.[8]				do	
18	Heart's blood of white mouse SM214 inoculated subcutaneously with culture on egg.	Aug. 14	Good growth.	No growth				do	
19	Heart's blood of white mouse SM215 inoculated subcutaneously with culture on egg.	do	Good growth, second day.	Growth, seventh day.[3]				do	

20	Heart's blood of white mouse SM217 inoculated subcutaneously with heart's blood of mouse.	July 17	.....do.....	No growth....	.....	.....	.....	.....do.....	
21	Spleen of rabbit RL20 inoculated subcutaneously with heart's blood of mouse SM206.	July 26	Good growth, third day.	Good growth, sixth day.[3]	Growth, fourth day.[6]	No growth....	Good growth, sixth day.[6]	.....do.....	
22	Spleen of rabbit RL26 inoculated subcutaneously with heart's blood of mouse SM206.	July 29	Good growth, sixth day.	.....do.[4].....	Good growth, third day.[6]	Good growth, ninth day.[6]	No growth....	.....do.....	No growth.
23	Spleen of rabbit RL3 inoculated subcutaneously with heart's blood of mouse SM206.	July 30	Growth, second day.	No growth....	Growth, third day.[6]	Growth, eighth day.[6]	Good growth, seventh day.[6]	.....do.....	
24	Spleen of rabbit RL24 inoculated subcutaneously with heart's blood of mouse SM206.	...do....	.....do.....	.....do.....	.....do.[5].....	Growth, third day.[6]	No growth....	.....do.....	Do.
25	Spleen of rabbit GCR inoculated subcutaneously with heart's blood of mouse SM206.	July 29	No growth....	.....do.....	Growth, fourteenth day.[6]	Growth, seventeenth day.[6]	.....do.....	.....do.....	Do.
26	Spleen of guinea pig vaccinated with spleen of mouse 30.	...do....	Good growth, fifth day.	Good growth, ninth day.	No growth....	No growth....	.....do.....	.....do.....	Do.
27	Spleen of guinea pig vaccinated with spleen of mouse 21.	Aug. 5	Good growth, second day.	Good growth, seventh day.[1]	.....do.....	.....	.....do.....	.....do.....	Do.
28	Spleen of guinea pig vaccinated with spleen of mouse C4.	Aug. 14	Good growth, third day.	Good growth, fourth day.[1]	Growth, fourth day.[3]	.....	Growth, twelfth day.[6]	.....do.....	Do.
29	Spleen of guinea pig vaccinated with spleen of mouse 31.	Aug. 30	Good growth, fifth day.	No growth....	Growth, seventh day.	No growth....	No growth....	.....do.....	Do.
	Total number of "growths" on each medium..	.....	23	21	22	9	11		
	Total number of "no growths" on each medium	.....	8	6	4	6	12		
	Percentage of "growths" on each medium.....	.....	74.2	77.7	84.6	60	48		

[1] Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen from second to fifth generations; the fifth generation was rubbed on the shaved abraded skin of a guinea pig causing its death acutely with typical lesions of tularæmia.

[2] "Vaccination" means that the shaved abraded skin was rubbed with a culture or with a piece of infected tissue.

[3] Subcultures on this same medium for the second, third, and fourth generations grew well.

[4] A subculture on this same medium for the second generation showed growth.

[6] A subculture on this same medium for the second generation failed to grow.

[7] Subcultured on serum glucose agar for the second generation and on serum glucose agar plus a piece of fresh sterile rabbit spleen for the third to sixth generations; the sixth generation rubbed on the shaved abraded skin of the guinea pig caused its death with typical lesions of tularæmia.

[8] A subculture on coagulated egg yolk grew well.